American Journal of Botany
American Journal of Botany 100(9): 1871–1882. 2013.

Propagule pressure, genetic structure, and geographic origins of *Chondrilla juncea* (Asteraceae): An apomictic invader on three continents[1]

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- *Premise of the study:* Assessing propagule pressure and geographic origins of invasive species provides insight into the invasion process. Rush skeletonweed (*Chondrilla juncea*; Asteraceae) is an apomictic, perennial plant that is invasive in Australia, South America (Argentina), and North America (Canada and the United States). This study comprehensively compares propagule pressure and geographic structure of genotypes to improve our understanding of a clonal invasion and enhance management strategies.
- *Methods:* We analyzed 1056 native range plants from Eurasia and 1156 plants from three invaded continents using amplified fragment length polymorphism (AFLP) techniques. We used measures of diversity (Simpson's D) and evenness (E), analysis of molecular variance, and Mantel tests to compare invasions, and genotype similarity to determine origins of invasive genotypes.
- *Key results:* We found 682 unique genotypes in the native range, but only 13 in the invaded regions. Each invaded region contained distinct AFLP genotypes, suggesting independent introduction events, probably with different geographic origins. Relatively low propagule pressure was associated with each introduction around the globe, but levels of among-population variation differed. We found exact AFLP genotype matches between the native and invaded ranges for five of the 13 invasive genotypes.
- *Conclusions:* Invasion dynamics can vary across invaded ranges within a species. Intensive sampling for molecular analyses can provide insight for understanding intraspecific invasion dynamics, which can hold significance for the management of plant species, especially by finding origins and distributions of invasive genotypes for classical biological control efforts.

Key words: AFLPs; Asteraceae; biological control; *Chondrilla juncea*; invasive; origins; propagule pressure; rush skeletonweed; weed.

The invasion process can be viewed as a series of steps in which propagules of a species (e.g., seeds, eggs, larvae, rhizome and stem fragments, mature individuals) are taken up from the native or already invaded range and transported to a new area (Kolar and Lodge, 2001; Sakai et al., 2001). Across the range of a widespread invasive species there may be dissimilarities in propagule pressure (founder population size and/or number of introduction events), genetic diversity, allocation of resources, levels of competitive ability, or resistance or tolerance to control methods including natural enemies, all of which have implications for ongoing invasion dynamics (e.g., Hänfling et al., 2002; Urban et al., 2008; Farrer et al., 2011; Robert et al., 2012). Propagule pressure is now recognized as a predictor of establishment success and the likelihood of invasion (Kolar and Lodge, 2001; Colautti and MacIsaac, 2004; Lockwood et al., 2009; Simberloff, 2009) and is determined by the propagule size, or propagule number, or both (sensu Simberloff, 2009). Propagule size is defined as the number of individuals in a propagule (founder population size), and propagule number is defined as the number of discrete introduction events (Simberloff, 2009). Whether the propagules come from one source or from different sources in a metapopulation ("propagule pool" vs. "migrant pool" sensu Slatkin (1977)) can have bearing on the genetic diversity of the invasion. Recent literature has focused on the importance of multiple introduction events during biological invasions, and a growing body of evidence suggests that multiple introductions may be the rule rather than the exception (Novak and Mack, 2001, 2005; Wares et al., 2005; Lavergne and Molofsky, 2007; Dlugosch and Parker, 2008; Kolbe et al., 2008; Wilson et al., 2009; Keller and Taylor, 2010; Estoup and Guillemaud, 2010).

With the number of biological invasions and their negative consequences expected to increase in the future, determining how, when, and from where invaders are introduced becomes crucial to gaining a better understanding of the role of propagule pressure in the invasion process (Novak, 2007; Wilson et al., 2009; Estoup and Guillemaud, 2010). Determining the propagule

[1]Manuscript received 2 December 2012; revision accepted 16 May 2013.

Special thanks to G. Markin for originally suggesting and enabling this research and to anonymous reviewers and the Editorial Staff at the *American Journal of Botany* for their valuable help. Many thanks to all collectors listed in Appendices S1 and S2 (see Supplemental Data with the online version of this article) and to K. Mann and J. Lassey for laboratory work. This research was made possible in part by funding from the USDI Bureau of Land Management (to J.F.G.) and the Committee for Research and Exploration of the National Geographic Society (to C.L.K., grant no. 7884-05).

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doi:10.3732/ajb.1200621

pressure of a biological invasion can be done using historical information (e.g., herbarium or museum specimens, or written accounts) that provides insights into the number of founders and/or the number of introduction events in the new range (Simberloff, 2009; Estoup and Guillemaud, 2010; Huttanus et al., 2011), but this information is often lacking for accidentally introduced plants. The manner in which a species is introduced into its new range (i.e., its propagule pressure) holds genetic consequences for invasive populations. Thus, molecular markers and population genetic parameters can be used to estimate the genetic signature of propagule pressure and identify the geographic origins of invasive populations (Estoup and Guillemaud, 2010; Novak, 2011). The use of molecular markers can also provide insight into the genetic variation of invasive populations that may be driven by founder effects, postintroduction hybridization, and/or natural selection (Ellstrand and Schierenbeck, 2000; Sakai et al., 2001; Lee, 2002; Petit, 2004; Goolsby et al., 2006a; Keller and Taylor, 2008; Le Roux and Wieczorek, 2009; Prentis et al., 2009). In addition, understanding the amount and distribution of genetic diversity within and among invasive populations can enhance the efficacy of management programs (Roderick and Navajas, 2003; Müller-Schärer et al., 2004; Strong, 2004; Gaskin et al., 2011). For example, determining the geographic origins of invasive genotypes can aid in the search for effective classical biological control agents, as natural enemies can be host-specific at the population or genotype level (e.g., Garcia-Rossi et al., 2003; Evans et al., 2005; Goolsby et al., 2006b).

Rush skeletonweed (*Chondrilla juncea* L., Asteraceae) is an invasive perennial herb native to Eurasia and North Africa (McVean, 1966; Panetta and Dodd, 1987) that primarily reproduces clonally via autonomous gametophytic apomixis, though there may be some residual sexuality in the native range (Chaboudez, 1994). This species has been accidentally introduced to and has invaded Australia, Argentina, Canada, and the United States (Schirman and Robocker, 1967; Cullen and Groves, 1977; Tortosa and Medan, 1977). In Australia, *C. juncea* is recognized as the most problematic weed of wheat-growing regions (Cuthbertson, 1967; Panetta and Dodd, 1987). Negative economic consequences include reductions of up to 80% in wheat yield (Panetta and Dodd, 1987) and damage to wheat harvest machinery (Cuthbertson, 1967). In Argentina, the species is considered a National Plague of Agriculture by the Fiscalización Fitosanitaria. In the northwestern United States, *C. juncea* now occupies approximately 2.5 million ha of croplands, grazing lands, and natural areas (Cuthbertson, 1967; Sheley and Hudak, 1995). In Idaho alone, the infested area has increased from 20 ha in the 1960s to 1.4 million ha in the mid 1980s, most notably in national forests (Piper and Andres, 1995).

Previous studies using morphological, phenological, and allozyme data indicated that *C. juncea* invasions consist of multiple introduced biotypes (Chaboudez, 1994; Hasan et al., 1995; McCaffrey et al., 1996) that have different susceptibility to certain biological control agents and herbicides (Burdon et al., 1984; Black et al., 1998; Campanella et al., 2009). For example, due to extreme host-specificity, imported strains of the fungal rust biological control agent *Puccinia chondrillina* Bubak. & Syd. are not effective against two of the three *C. juncea* biotypes in Australia, one of the three biotypes in the United States, and have had little effect on plants in Argentina. In Australia, the two rust-resistant biotypes of *C. juncea* have extended their distribution to replace the susceptible biotype (Burdon et al., 1981). For these and other reasons, biological control of *C. juncea* has not been considered successful on any invaded continent (Burdon et al., 1981; Prather, 1993; Vigna et al., 1993; Milan et al., 2006).

Chondrilla juncea possesses a wide geographic distribution in its native range (McVean, 1966; Panetta and Dodd, 1987), but many native range regions have not been sampled or had plants analyzed with genetic markers. In addition, *C. juncea* biotypes in Canada, western Australia, and eastern North America invasions have not been identified, and there has been no comprehensive comparison of *C. juncea* genotypes among the three invaded continents. Morphological and phenological biotypes of *C. juncea* are difficult to identify unless plants are grown under common garden conditions (Hill and Groves, 1973). Allozyme techniques were used to identify three biotypes in Australia, three in the United States, and three in Argentina, and plants from Australia and the United States have been compared with plants from a portion of the native range: the former Yugoslavia, Italy, Greece, and Turkey (Chaboudez et al., 1992; Hasan et al., 1995). However, the use of allozymes generally underestimates genetic variability compared with more recently developed marker systems such as AFLPs, simple sequence repeats (SSRs) and inter-simple sequence repeats (ISSRs) (Cabrita et al., 2001; Ipek et al., 2003; Le Roux and Wieczorek, 2009).

The goal of this study was to improve our understanding of the *C. juncea* invasion. We hypothesized that low propagule pressure (i.e., few genotypes and few introduction events) of this invasion led to strong geographic structuring on a regional basis. We also hypothesized that the use of highly polymorphic molecular markers such as AFLPs, along with more comprehensive sampling, will provide a more complete inventory of native and invasive genotypes, improving our ability to find invasion origins. We will discuss the implications of these results on future biological control efforts directed toward *C. juncea* and other clonally propagating invasive plant species.

MATERIALS AND METHODS

Plant collections—Ligules (petal-like corollas of ray florets found in composite flowers) were collected because earlier attempts at DNA amplification from *C. juncea* leaf tissue were unsuccessful, possibly due to secondary compounds associated with the latex in vegetative portions of these plants. To avoid foreign pollen contamination, collectors endeavored to collect flowering heads just prior to their opening. Each flower head remains open for approximately 1 d (Panetta and Dodd, 1987), though on any given day, multiple flower heads can be open on a plant. Collectors did not obtain samples from plants closer than 5 m apart to avoid collecting ramets connected by underground rhizomes.

We sampled a total of 2206 plants on five continents. In the native range, we sampled 1050 plants from 149 populations in 21 countries ranging from Spain to Uzbekistan (Fig. 1; Appendix S1, see Supplemental Data with the online version of this article). The final number of plants sampled from each native population varied from 1 to 20, with an average (±SD) of 7.0 ± 4.3 plants per population. In the invaded range, we sampled 1156 plants from 154 populations (North America: 721 plants, 96 populations; Australia: 377 plants, 52 populations; Argentina: 58 plants, six populations; Figs. 2, 3A–C, Appendix S2, see online Supplemental Data). The final number of plants sampled from each invasive population varied from 1 to 20, with an average of 7.5 ± 3.9 plants per population. The number of populations sampled was higher in putative areas of original introduction such as the vicinity of Wagga Wagga, New South Wales, Australia and Spokane, Washington, United States, and in densely invaded areas such as southern Idaho, and the central California foothills, United States. We sampled relatively few populations in Argentina as the invasion is limited to an area approximately 200 × 300 km, and also in western Australia and the eastern United States, where *C. juncea* is subject to a rigorous eradication program or is less common, respectively. We also genotyped Australia's Commonwealth

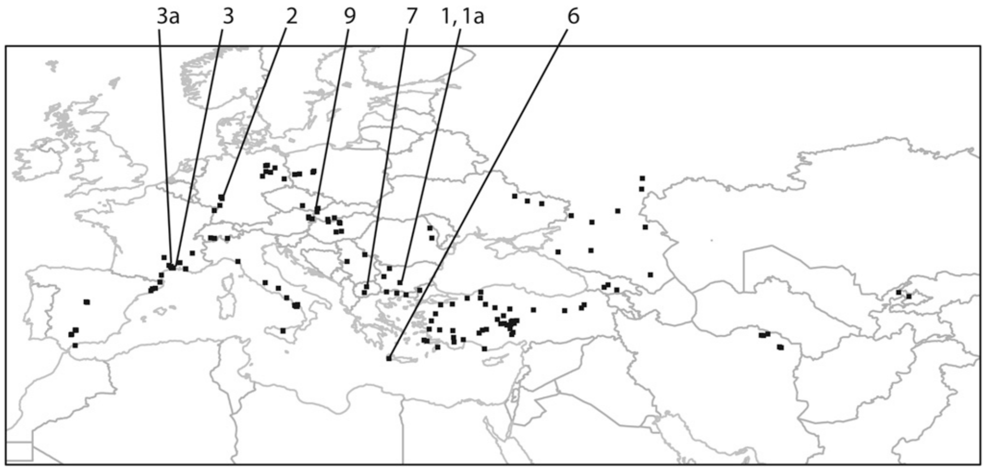

Fig. 1. Map of sampled *Chondrilla juncea* L. populations in Eurasia. Lines connect populations containing individuals showing ≥0.95 similarity to invasive genotypes from North America (1, 1a, 2, 3, 3a) and Australia (6, 7). Other invasive genotypes had <0.95 similarity with a native genotype and are not shown.

Scientific and Industrial Research Organization (CSIRO) seed collections identified as the three biotypes found in Australia (N = 3 for each biotype).

DNA extraction and AFLP analysis—DNA was extracted from approximately 3 mg of silica-dried ligules using the modified CTAB method of Hillis et al. (1996). AFLP analysis followed Vos et al. (1995) with modifications as described in Gaskin and Kazmer (2009). Loci were scored with the program GeneMapper v 4.0 (Applied Biosystems, Foster City, California, USA). All selective primer combinations of MseI + CAA, CAC, CAT, CTA, or CTC and EcoRI + AAG, ACC, or ACT were prescreened for eight samples and the two most polymorphic primer pairs were chosen (MseI + CAC/EcoRI + ACT and MseI + CTC/EcoRI + ACC).

Error checking of AFLP data and number of genotypes—To determine geographic origins and propagule pressure, we needed to account for AFLP error, then identify the number and similarity of genotypes in the native and invaded ranges. To test for AFLP error rate, we repeated the AFLP procedure using 48 plants starting at the restriction/ligation stage, for each primer pair. In addition, all plants with unique genotypes (initially N = 76) in the invaded range were reanalyzed for AFLPs (starting at the restriction/ligation stage) to check for AFLP artifacts. If we obtained the same genotype upon reanalysis, the genotype was considered to be error-free. Reanalyzing AFLPs on unique genotypes in the native range (initially N = 965) was deemed too costly a process, so we used the software GenoType (Output option = *Genotypes file*; Meirmans and Van Tienderen, 2004) to estimate number of unique genotypes. This software asks for a threshold value (error rate) that indicates the maximum number of differences in alleles that is allowed between two individuals to still be considered the same genotype. The threshold options are only available as integers, so we chose the threshold of three alleles, which was closest to our calculated AFLP error rate of 2.8 alleles per plant genotype (see Results below).

Genotypic similarity—This is the only analysis for which we incorporated the error rate. NTSYS-PC ver. 2.1 software (Rohlf, 1994) was used to calculate the Dice pairwise similarity coefficient: $2a/(2a + b + c)$, where a = number of bands present in both samples, b and c = number of bands present in only one or the other sample. This coefficient considers that when both samples are missing a band, the band absences are not necessarily comparable. To visually assess the diversity of invasive genotypes, we used Dice similarity coefficients to create a UPGMA dendrogram of invasive and native AFLP genotypes using the SAHN module of NTSYS.

Genetic diversity and geographic structure of genotypes—To determine how different genotypes are geographically distributed in the invasive and native ranges, we first used two measurements: Simpson's diversity index (D), corrected for finite sample size (Pielou, 1969), was calculated as $D = 1 - \Sigma n_i(n_i - 1)/N(N - 1)$ for i = 1 to G, where n_i is the number of plants that share genotype i, G is the number of unique genotypes, and N is the total number of plants, with values of D ranging from 0 to 1 and higher values corresponding to greater genetic diversity. Genotypic evenness (E) (Fager, 1972) was calculated with $E = (D - D_{min})/(D_{max} - D_{min})$, where $D_{min} = (G - 1)(2N - G)/N(N - 1)$ and $D_{max} = N(G - 1)/G(N - 1)$, with values of E ranging from 0 to 1, and lower values indicating that a certain genotype is common, and higher values indicating that the number of plants representing each genotype is more evenly distributed.

To assess how genetic variation is distributed within vs. among populations and regions, we used analysis of molecular variance (AMOVA), as implemented in the program Arlequin version 3.5.1.2 (Excoffier et al., 1992). For some populations, we collected only a few individuals; thus, we assessed the bias of omitting under-collected populations by performing AMOVA both with and without populations containing fewer than five individuals.

For both the native and invaded ranges, we determined the correlation between genetic and geographic distances using a Mantel test. To obtain genetic distances between populations, we used the statistical software R v 2.13.0 (R Development Core Team, 2011) with the Hickory function from AFLPdat (Ehrich, 2006) to convert AFLP data into the correct format for Hickory v1.1 (Holsinger et al., 2002). Using default parameters in Hickory for burn-in, sampling, and thinning, we used the option that does not assume Hardy–Weinberg equilibrium (the *f*-free model) to estimate F_{IS}. The F_{IS} estimates (Eurasia, 0.497; North America and Argentina, 0.499; Australia, 0.498) were incorporated into the program AFLP-surv (Vekemans, 2002) to determine Nei's genetic distance (Nei, 1972), with Lynch and Milligan's (1994) correction, between populations. Estimates of allelic frequencies were computed with the fourth option in AFLP-surv; "Bayesian method with non-uniform prior distribution of allele frequencies". Nei's genetic distances between populations were compared to geographic distances between populations (calculated from decimal-degree coordinates using the AFLPdat function Geodist in R) in a Mantel test using the program IBD v 1.53 (Jensen et al., 2005) with 1000 permutations. Mantel tests were performed on what we considered contiguous ranges of *C. juncea* (Eurasia, western North America, eastern Australia, western Australia, and Argentina).

Geographic origins—Determination of geographic origins of genotypes in the invaded range was accomplished by indentifying highest Dice similarities between invasive and native plants. Because of the importance of error-free data in determining the geographic origins of invasive genotypes, we also manually compared (by visually overlapping) GeneMapper electropherograms to avoid software errors in peak-calling, especially errors of not calling low intensity peaks (e.g., relative fluorescence units: rfu < 50), which can occur due to the necessary filtering of noise in some electropherograms. This comparison was performed on all native plants that had > 95% Dice similarity to an invasive genotype.

RESULTS

AFLP error rate and number of genotypes—From the two pairs of AFLP primers, we selected 121 variable loci for the

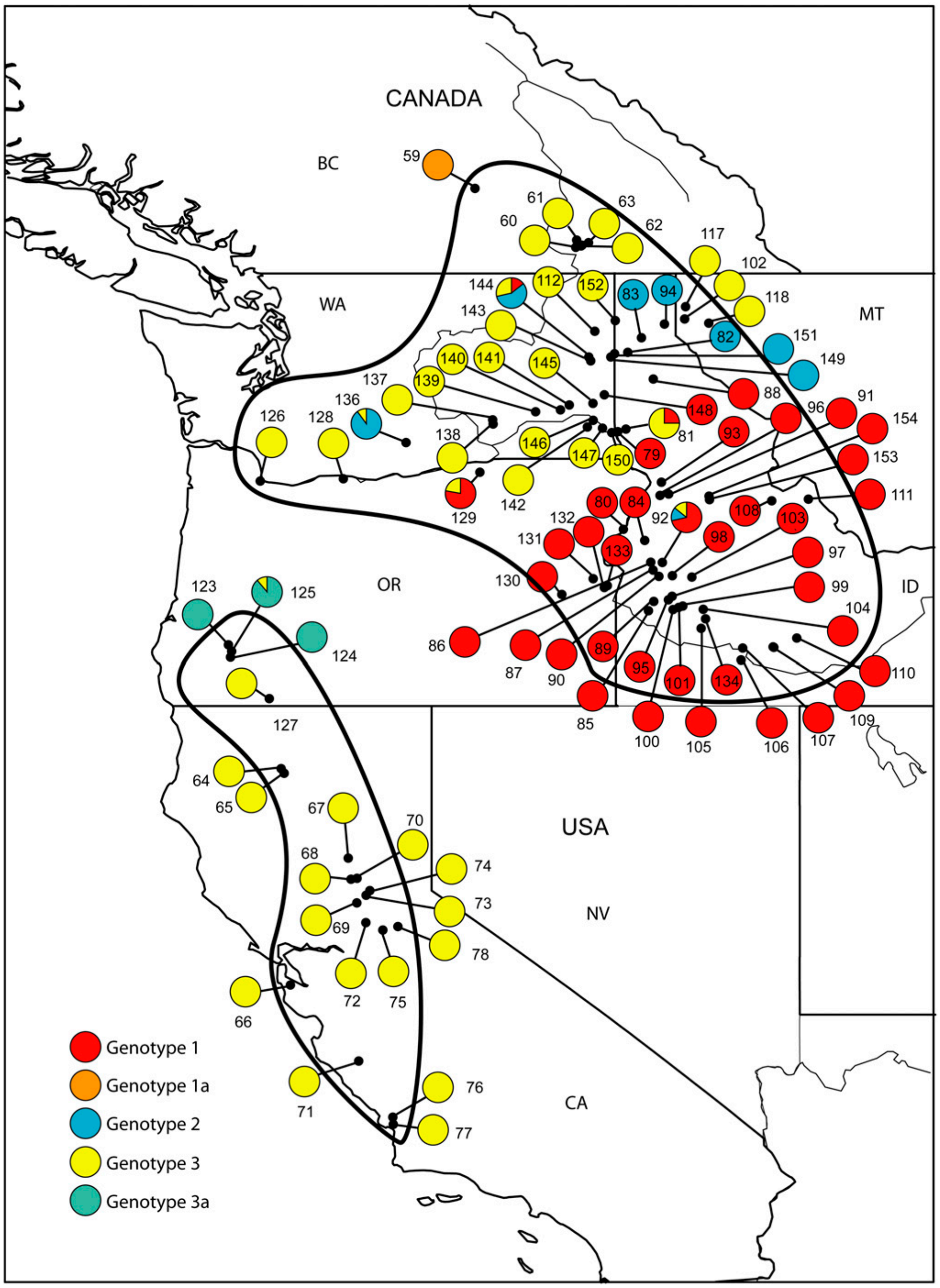

Fig. 2. Map of sampled *Chondrilla juncea* L. populations in western North America. Population identification number is inside, or next to, each circle. Circles of same color are the same genotype. Our estimates of current contiguous distributions of populations are shown by solid lines based on USDA (2005), Rice (2005), Calflora (2008), WeedMapper (2009), herbarium collections, and our personal field observations.

2206 *C. juncea* plants analyzed in this study. AFLP data are shown in Appendix S3 (see Supplemental Data with the online version of this article). There were 129/5808 mismatched AFLP calls for the 48 repeated plants, equaling a 2.2% (2.8/121 loci per plant) error rate.

In the native range, assuming that plants are actually identical genotypes if their alleles differed at a maximum of three AFLP loci, we found 682 unique genotypes (*G*) among the 1050 plants analyzed, with 576 singletons and 474 plants that were genetically identical to one or more other plants. One of the 149 native range populations was monotypic (data not shown). The number of clones (plants) for each genotype was n = 45, 39, 33, 15, 14, 13; all other genotypes consisted of fewer than 10 clones. We were able to distinguish 13 AFLP genotypes among the three invaded regions, with seven in North America (genotypes 1, 1a, 2, 3, 3a, 8, 9) and three each in Australia (genotypes 5, 6, 7) and Argentina (genotypes 4, 4a, 4b) (Figs. 2, 3A–C; Table 1). An "a" or "b" after the genotype number indicates that the genotype was very similar (≥0.95 Dice similarity) to a genotype designated by an integer only; e.g., genotypes 3

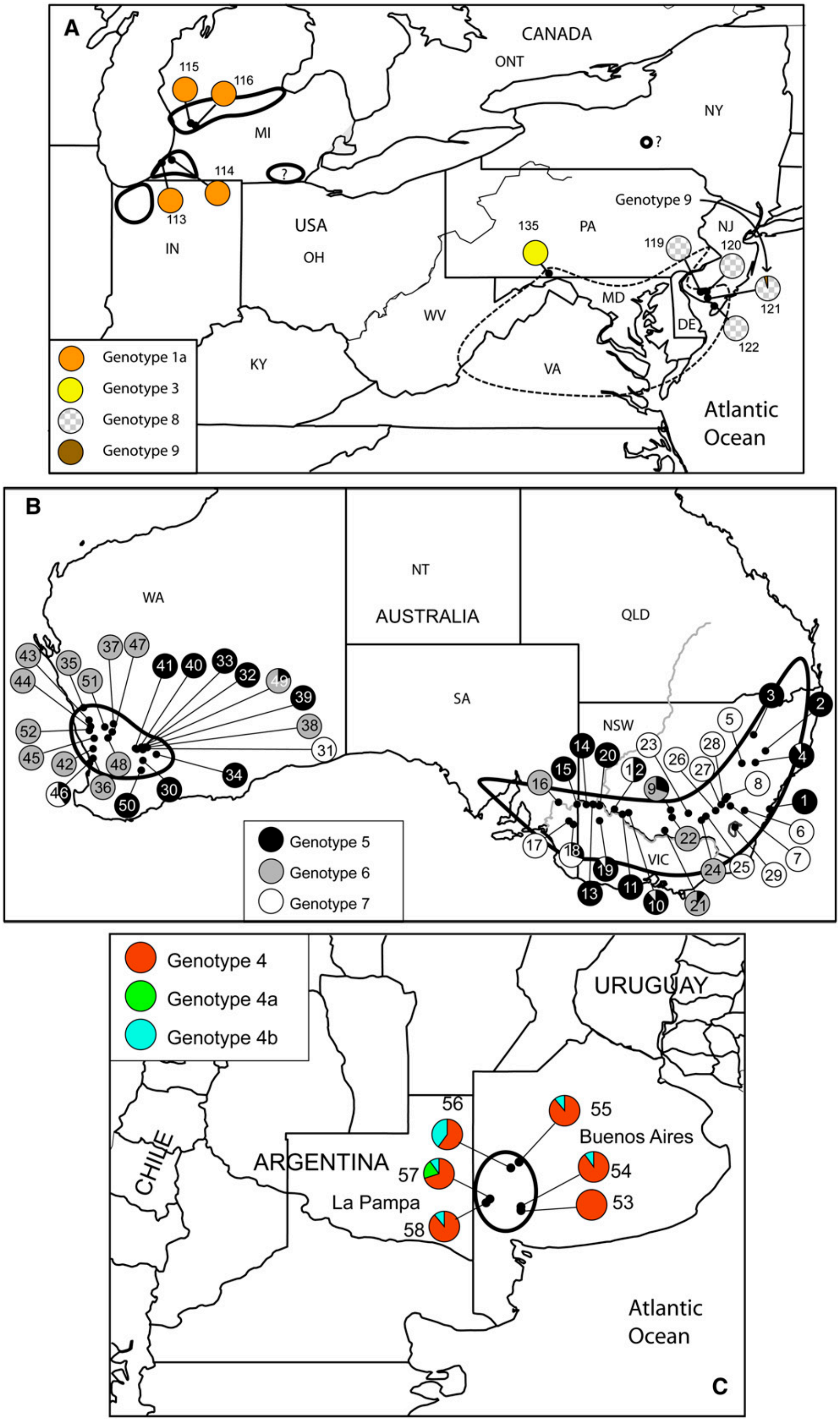

Fig. 3. Maps of sampled *Chondrilla juncea* L. populations in (A) midwestern and eastern North America, (B) Australia, and (C) Argentina. Population identification number is inside, or next to, each circle. Circles of same color are the same genotype, and no genotypes are shared among invasions on different continents. Our estimates of current contiguous distributions of populations are shown by solid lines, or dashed lines for historical (and probably extinct) distributions, and are based on Hill and Groves (1973), Vigna and Lopez (1989), USDA (2005), Rice (2005), Calflora (2008), A.V.H. (2009), WeedMapper (2009), herbarium collections, and our personal field observations.

TABLE 1. Number of invasive *Chondrilla juncea* plants sampled and their AFLP genotypes.

Region	1	1a	2	3	3a	4	4a	4b	5	6	7	8	9	Total
Argentina						48	2	8						58
Australia														
Western Australia									49	53	8			110
Eastern Australia									104	47	116			267
North America														
Western North America	208	20	99	278	27									632
Midwestern North America		32												32
Eastern North America				7								49	1	57
Total	208	52	99	285	27	48	2	8	153	100	124	49	1	1156

and 3a had ≥0.95 similarity (online Appendix S4). Even though we found an AFLP error rate of 2.2%, we did not combine these very similar invasive genotypes into one, as each genotype was represented by multiple plants or repeated for AFLP analysis (Table 1), confirming that these AFLP banding differences were not artifacts. Most invaded range populations were monotypic (North America 93%, Australia 83%) except in Argentina (20%) (Figs. 2, 3A–C).

Genetic similarity—Average Dice similarity (not corrected for AFLP error) between native plants was 0.66 ± 0.08. As indicated by their positions throughout the UPGMA dendrogram (Fig. 4), genotypes in Australia were relatively dissimilar (Dice similarities 0.67–0.74; Appendix S4), while Argentinean genotypes clustered together (Dice similarities 0.95–0.99). In North America some genotypes were similar to each other (1 and 1a; Dice similarity 0.98 and 3 and 3a; Dice similarity 0.99), whereas other genotypes (2, 8 and 9) were less similar (as low as 0.66 Dice similarity).

Genetic diversity and geographic structure of genotypes—Diversity in the native range was high (D = 0.99), and the number of plants representing each genotype was relatively even (E = 0.96) (Table 2). Diversity was lower in the invaded ranges (D = 0.73–0.30). Evenness was higher in Australia and North America than in Argentina, due to the low frequencies of genotypes 4a and 4b in Argentina.

None of the AFLP genotypes were found on more than one invaded continent. The two most common genotypes in North America were 3 (285/721 = 40%) and 1 (208/721 = 29%), followed by 2, 1a, 8, 3a, and 9 (Table 1; Figs. 2, 3A). Four genotypes were detected in the midwestern and eastern United States (1a, 3, 8, and 9) and five genotypes in western North America (1, 1a, 2, 3, and 3a). Notably, California contained only genotype 3 and the southern Idaho/eastern Oregon region was dominated by genotype 1 (99% of 178 plants). Australia contained three genotypes (5, 6, and 7; Fig. 3B), with frequencies of 45%, 48%, and 7%, respectively, in western Australia, and 39%, 18%, and 43%, respectively, in eastern Australia. In Argentina, the most common genotype was 4, found in all populations, followed by 4b and 4a (Table 1; Fig. 3C), with frequencies of 83%, 14%, and 3%, respectively.

The AMOVA of native populations indicated that 47% of genetic variation was among populations, and 52% was within populations (P < 0.001; online Appendix S5). The AMOVA of Argentina samples showed little differentiation (7.4%, P = 0.08) among populations, whereas all other invaded regions and contiguous invasions contained 87–96% of variation among populations (P < 0.001), except for the central United States, which was monotypic. We also performed AMOVAs on sets of data with populations containing fewer than five individuals included (data not shown). Percentages of variation among or within populations did not vary by more than 1% from the results shown in Appendix S5.

The Mantel tests indicated significant and relatively moderate correlations between genetic and geographic distances among native populations (r = 0.34, P < 0.01). There were significant and relatively moderate correlations between genetic and geographic distances for western North America (r = 0.28, P < 0.001) and western Australia (r = 0.36, P < 0.001) and a lower but significant correlation for eastern Australia (r = 0.14, P = 0.014). For Argentina, there was no significant correlation (r = 0.20, P = 0.331).

Geographic origins—We were able to find genetically identical AFLP matches in the native range for five of the 13 invasive genotypes (Fig. 1). Additionally, three invasive genotypes matched native genotypes at ≥0.95 Dice similarity, and five genotypes matched at 0.83–0.94 similarity. The two native populations containing plants with highest Dice similarities to each invasive genotype are reported in Table 3.

DISCUSSION

Propagule pressure and population structure—The large number of genotypes in the native range (682) compared to the low number of invasive genotypes (13) detected across the three invaded continents suggests relatively low propagule pressure, which has resulted in severe founder effects for these invasions. In addition to a low number of genotypes, we also see low genotype diversity (Simpson's D) and high genetic structure in most invaded regions. Taken together, these data suggest that few native genotypes were sampled, and few introduction events took place, to form these founding populations.

The presence of only one native population that was monotypic was surprising in comparison with the many monotypic populations in North America and Australia. Chaboudez (1994) found high diversity among populations in Turkey (91 genotypes across 123 populations). Along with our finding of only a single monotypic population, that suggests that sexual reproduction in the native range may be more common than previously suspected. The low to moderate correlation between genetic distance and geographic distance suggests gene flow decreases with distance across the native range. This also appears to be true for North America and Australia, whereas gene flow does not appear to be limited in the relatively small geographic area invaded by *C. juncea* in Argentina.

The majority of genetic variation in North America is distributed among populations as indicated by the AMOVA results

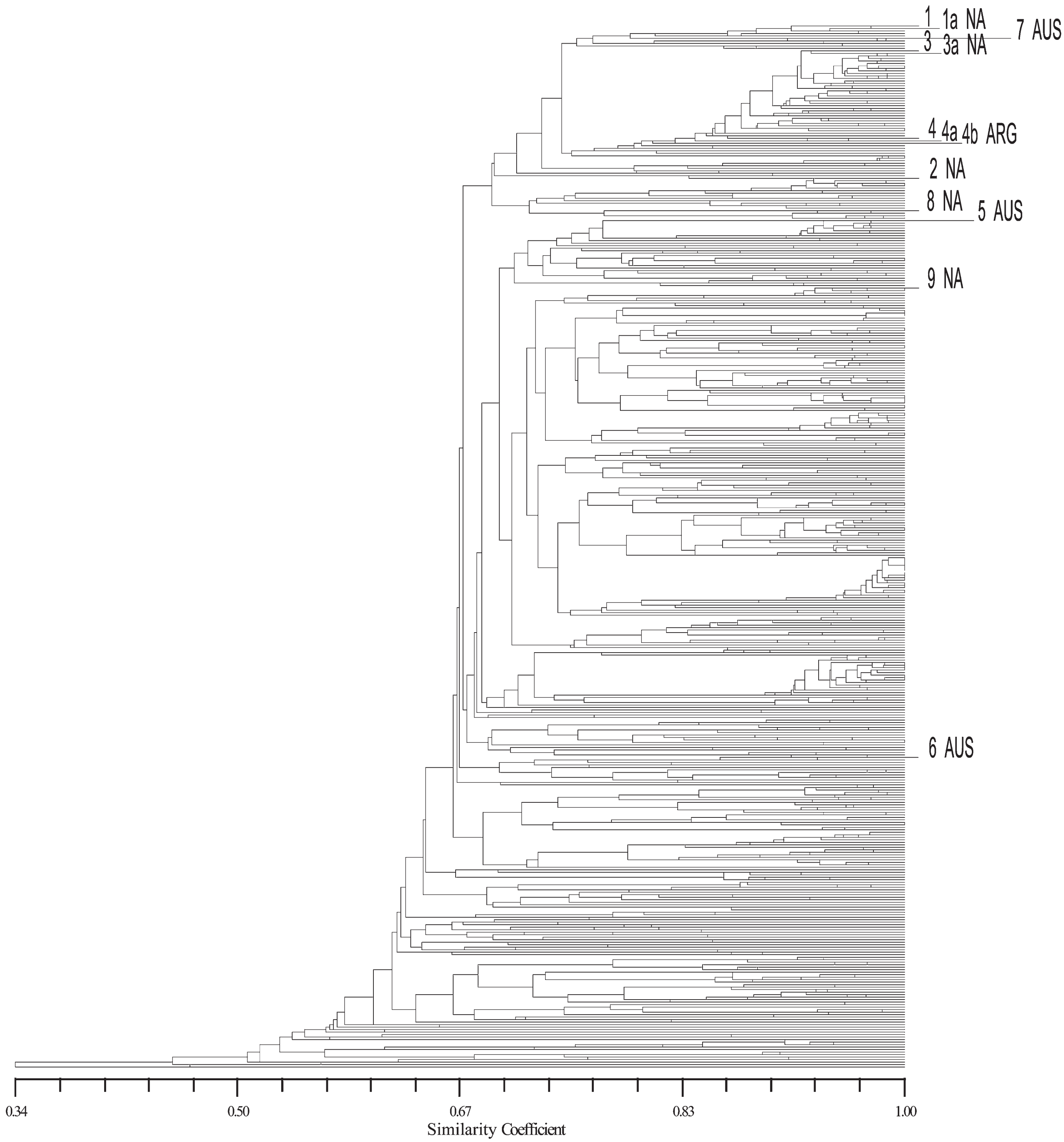

Fig. 4. A UPGMA dendrogram of 1050 native and 13 invasive *Chondrilla juncea* plant genotypes based on Dice similarities for AFLP genotypes from Eurasia, Argentina, Australia, and North America. Invasive genotypes are indicated, followed by their invasive range (NA = North America, AUS = Australia, ARG = Argentina). All unlabeled "tips" are native genotypes.

and high percentage of monotypic populations, suggesting that, barring the extirpation of genotypes through stochastic events or natural selection, the few population founding events usually contained single propagules or multiple genotypically identical propagules from the same source population. In addition, our data suggest low propagule pressure from genetically distinct established populations during subsequent range expansion. The same pattern appears to hold for Australian populations, where genetic variation is mostly partitioned among populations. Thus, Australian populations also appear to have been

TABLE 2. Number of *Chondrilla juncea* genotypes and diversity and evenness of genotype distribution for different continents and regions.

Region	No. of plants sampled	*G*	*D*	*E*
Eurasia	1050	682	0.99	0.96
Argentina	58	3	0.30	0.38
Australia	377	3	0.66	0.98
Western Australia	110	3	0.57	0.88
Eastern Australia	267	3	0.63	0.94
North America (NA)	721	7	0.73	0.84
Western NA	632	5	0.67	0.84
Midwestern NA	32	1	0	
Eastern NA	57	3	0.25	0.30

Notes: G, number of genotypes found in region; *D*, Simpson's diversity index corrected for finite sampling; *E*, Evenness.

established following low propagule pressure (i.e., from single or multiple genetically homogenous propagules). In Argentina, the opposite pattern is observed, where genetic variation is found mostly within populations, but still propagule pressure appears low, with few genotypes introduced, and these are likely from a single introduction event and origin since they are genetically very similar.

***Comparison to earlier studies*—**In western North America AFLP genotypes 1 and 1a correlate with the "Banks" biotype, genotype 2 with the "Washington early-flowering" biotype, and genotypes 3 and 3a, with the "Washington late-flowering" biotype (Schirman and Robocker, 1967; Rosenthal et al., 1968; Lee, 1986; Hasan et al., 1995; McCaffrey et al., 1996). Our wider sampling increased the known number of invasive genotypes described in North America from three to seven, with two of these (8 and 9) being genetically distinct from previously identified genotypes. The new genotypes (8 and 9) found in the eastern United States have no previous biotype designations.

Three biotypes and three allozyme genotypes had previously been reported for Australia (Burdon et al., 1980), and we did not find any additional genotypes. Our AFLP genotypes correlate with Australian biotypes as follows: genotype 5 = Form C-broad rosette leaves; 6 = Form A-narrow rosette leaves; 7 = Form B-intermediate width rosette leaves. Hill and Groves (1973) suggested that Form A once had the most widespread distribution in eastern Australia, from southeastern Queensland to the New South Wales/Victoria border, and west into South Australia. Form B and Form C were found to be limited to east-central New South Wales. Burdon et al. (1981) found that Forms B and C had increased their range since the 1973 study, probably because biological control efforts created a decline in the frequency of Form A in central New South Wales (Fig. 3B). In contrast, we find that all three AFLP genotypes are relatively common and evenly represented in eastern Australia. These findings may be explained by two scenarios: (1) Form A was not reduced by biological control methods as suggested by Burdon et al. (1981), or (2) Form A may have increased in abundance since the 1981 study despite biocontrol efforts. Additional research will be required to assess which of these scenarios explains our result. In a previous study in Argentina (Sacco, 1988), it was unclear how the common and rare allozyme genotypes were distributed within and among populations of this invasion, so we can make no comparisons to our present data.

***Geographic origins*—**In the native populations, we found approximately double the number of genotypes found in an earlier study (682 genotypes from 1050 plants [65%] compared to 326 genotypes from 983 plants [33%]; Chaboudez et al., 1992), suggesting that more intensive plant sampling in the native range and the use of AFLP markers improved our fine-scale knowledge of

TABLE 3. Highest Dice pairwise similarity coefficients between native and invasive *Chondrilla juncea*. Highest match of an invasive genotype to a native genotype is followed by highest match to a native genotype from an alternate population.

Invasive genotype	Invasion	Dice similarity	Country	Pop. no.	Individual no.
1	North America	0.99	Bulgaria	7	236
		0.97	Macedonia	86	860
1a	North America	1.00	Bulgaria	7	235
		0.97	Macedonia	86	860
2	North America	1.00	Germany	43	425
		0.99	Spain	124	401
3	North America	1.00	France	27	230
		1.00	Spain	112	173
3a	North America	1.00	France	15	41
		0.99	Spain	112	173
4	Argentina	0.94	France	22	176
		0.91	Spain	111	165
4a	Argentina	0.94	France	22	176
		0.93	Spain	111	165
4b	Argentina	0.94	France	22	176
		0.89	Italy	69	287
5	Australia	0.83	France	18	738
		0.82	Spain	115	363
6	Australia	0.96	Greece	61	1050
		0.87	Russia	100	977
7	Australia	0.99	Macedonia	85	851
		0.88	Romania	92	335
8	North America	0.86	France	19	102
		0.85	France	27	230
9	North America	1.00	Slovakia	104	69
		1.00	Hungary	62	79

Notes: For population location and GPS coordinates, see Appendix S1.

invaded range origins. Our data indicate that populations of *C. juncea* in North America, Australia, and Argentina do not share genotypes, suggesting that the introductions into these three regions were independent events, probably with different geographic origins, as has been found in other invasions (e.g., Besnard et al., 2007; Reusch et al., 2010).

To determine geographic origins of invasive genotypes, it is helpful, but not required, for native populations to possess significant genetic structure (Keller and Taylor, 2008; Novak, 2011). If genetic diversity is highly structured, genotype comparisons may be used to pinpoint origins to a specific population (e.g., the invasion came from population A, and most individuals in an invasive population are genetically similar to individuals in population A (e.g., Novak and Mack, 2001; Goolsby et al., 2006a). If genetic diversity is less structured, origins may not be linked to a specific population but may be associated with a certain region in the native range (e.g., Milne and Abbott, 2004). In our study, we were able to describe exact matches between native and invasive AFLP genotypes for five of the 13 invasive genotypes. In addition, we found high genetic similarity (>0.95) for three other genotypes. Identifying the geographic origins of an invasive is not only facilitated by high genetic structure, but increases in accuracy with more intensive sampling of native populations (Muirhead et al., 2008). In addition, the probability of identifying the geographic origins of an invasion is much higher for species with relatively low genetic diversity, such as self-pollinating plants and species that reproduce through asexual (clonal) means (Novak and Mack, 2001; Facon et al., 2003; Novak, 2011).

We found Dice similarity matches between invasive genotypes and individual native plants that differ from assessments of geographic origins previously described. In earlier work, plants from the former Yugoslavia, Italy, Greece, and Turkey (Chaboudez et al., 1992; Chaboudez, 1994; Hasan et al., 1995) were analyzed, and Hasan et al. (1995) found allozyme genotypes in Yugoslavia and Greece that matched two of the three patterns found in the USA (our genotypes 1 and 3, and perhaps 1a and 3a). Hasan et al. (1995) also suggested that the genetic relationships among native and invasive populations might be further explored by sampling plants more broadly across Eurasia and using a more polymorphic genetic marker. Just such an approach was taken in our study. In general, we found the best genotype matches in different regions of the native range compared with the regions reported in Hasan et al. (1995). For genotypes 1 and 1a, our best match occurred in Bulgaria, which is geographically close to their matches from Yugoslavia and Greece, but for genotypes 3 and 3a, our best matches occurred in populations from western Europe (France and Spain) instead of their match in Yugoslavia. We also found a 100% match in Germany for genotype 2, for which there was no previous native range allozyme match.

Chaboudez et al. (1992) focused on comparisons of Australian genotypes with plants from former Yugoslavia, Italy, Greece, and Turkey. Later, Chaboudez (1994) focused solely on collections from Turkey. Neither study found an exact match between native and invasive populations using allozymes. We also failed to find an exact match for Australian genotypes 5, 6, and 7, but did find a 0.95 match in Greece for genotype 6 and a 0.99 match in Macedonia for genotype 7. Although we sampled heavily in Turkey (Fig. 1), we did not find any Dice similarities high enough to imply that this country is the origin of Australia's *C. juncea*.

Implications for biological control—Some present *C. juncea* biological control agents are host-specific at the genotypic level (Emge et al., 1981; Cullen and Moore, 1983) and thus not effective on all genotypes associated with an invasion. The discovery of identical or highly similar AFLP genotypes between the invasive and native ranges will allow future testing to determine whether the most highly coevolved agents (those found on plants with the same genotype) will be the most effective biological control agents (but see Hokkanen and Pimentel [1989]) for a discussion of "new associations").

In western North America, we found that certain genotypes dominate certain areas of the invasion. Though the outcomes of biological control efforts are dependent on many factors, including life history, genetics of the control agent and environmental conditions (Chaboudez and Sheppard, 1995), if the invasion in a certain region is genetically monotypic (e.g., California) or nearly monotypic (e.g., southern Idaho), the regional invasion may be easier to control if an effective agent is found (Müller-Schärer et al., 2004).

While we did not find any additional genotypes in Australia, our study provides a better description of the geographic distribution of genotypes, allowing for more precise application of biological control agents. In western Australia, genotypes had not previously been identified, and based on our results genotype 6 is the most common (Fig. 3B; Table 1). This is the only Australian genotype susceptible to the rust *Puccinia chondrillina* released in eastern Australia, suggesting that the use of that agent in western Australia may be effective in the future. Our inability to find closer matches for genotype 5 and 6 suggests that the origins for these genotypes remain unsampled and may possibly occur in North Africa or further east in Asia.

In Australia and North America, the large amount of genetic variation partitioned among populations and the lack of genetic similarity between many genotypes poses the challenge of finding highly host-specific biological control agents for each genotype or finding an agent that is effective against all invasive genotypes in these regions. The control of only a subset of genotypes present in a population or region may lead to expansion of the more tolerant/resistant genotypes (Burdon et al., 1981), i.e., "self-defeating biological control" (Garcia-Rossi et al., 2003).

Currently, Argentina has used biological control agents developed for Australia and/or the USA, and foreign exploration for rust strains with high specificity to any of Sacco's (1988) three allozyme genotypes has not occurred. We failed to find high similarity matches (>0.95) for Argentina genotypes, indicating that the geographic origins of this invasion are undetermined. However, our results do point to France or Spain as regions for more intensive population sampling. Because the Argentina invasion encompasses a relatively small area compared to the invasions in North America or Australia, and most populations are mixtures of genetically similar AFLP genotypes (0.97–0.99 similarity), it is possible that all three genotypes will respond similarly to biological control agents (Sacco, 1988), but we suggest that all three genotypes should be included in any potential agent testing.

Conclusions—Highly variable AFLP markers are useful for distinguishing between lineages of clonal invasive species such as *C. juncea*. Only a small number of invasive genotypes (n = 13) were detected across the three invaded continents, compared with 682 genotypes across all native populations. This result alone indicates relatively low propagule pressure for invasion. Through the consistent use of the same molecular marker system across the entire range of *C. juncea*, we were able to find the geographic origins for many genotypes. In addition, we have detected differences in levels of AFLP diversity, evenness, and genetic structure of invaded regions and contiguous invasions. In some instances the use of more comprehensive population sampling and AFLP analysis produced results that mirror those previously obtained using allozymes (genotypes or biotypes introduced into Australia), and in other instances our study provided additional information (seven rather than three genotypes in North America).

Results of this study should enhance coordinated, multinational efforts to control this important invasive species and should be considered a useful addition to a classical biological control program. Identification of all genotypes in an invasion, which is likely possible in many clonally reproducing species, allows more complete testing of the host-specificity of potential biological control agents, thus lowering the risk that there will be unexpected resistance or tolerance to introduced biological control agents.

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